

U.S. Ser. No. 09/673,779 -7-
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WHAT IS CLAIMED IS:

1. (Amended) A method for identifying the presence of a bacterium in a sample comprising
 - a) testing said sample by Gram-staining and
 - b) testing said sample with a probe according to an *in situ* hybridisation protocol selected on the basis of the outcome of said Gram-staining and identifying the presence of the bacterium in the sample.
2. A method according to claim 1 wherein said sample is a clinical sample.
3. (Amended) A method according to claim 2 wherein said sample is mammalian blood.
4. (Twice Amended) A method according to claim 1 when said Gram-staining indicates the presence of a Gram-negative bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.
5. (Amended) A method according to claim 4 wherein said character is of the rod type, further comprising hybridising said sample with at least one probe selected from a group consisting of probes capable of hybridising with nucleic acid found *Escherichia coli*, in *Klebsiella pneumoniae*, in *Klebsiella oxytoca*, in *Serratia marcescens*, in *Enterobacter aerogenes*, in *Enterobacter cloacae*, in *Proteus vulgaris*, in *Proteus mirabilis*, in *Salmonella typhi*, in *Pseudomonas aeruginosa*.

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7. (Twice Amended) A method according to claim 6 wherein said probe is having no more than five mismatches with a probe selected of a group consisting of probes having a sequence GCCTGCCAGTTTCGAATG (SEQ ID NO:1) or GTAGCCCTACTCGTAAGG (SEQ ID NO:2) or GAGCAAAGGTATTAACCTTACTCCC (SEQ ID NO:3) or GTTAGCCGTCCCTTCTGG (SEQ ID NO:4).
8. A method according to claim 4 wherein said character is of the coccus type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.
9. (Twice Amended) A method according to claim 1, when said Gram-staining indicates the presence of a Gram-positive bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.
10. A method according to claim 9 wherein said character is of the rod type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme and/or Proteinase K.
11. (Amended) A method according to claim 9 wherein said character is of the coccus type, further comprising determining a chain-like or clump-like character of said bacteria.
12. A method according to claim 11 wherein said character is chain-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.
13. (Amended) A method according to claim 12 further comprising hybridising said sample with at least one probe selected from a group consisting of probes capable of

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14. A method according to claim 13 wherein said nucleic acid is ribosomal RNA.
15. (Twice Amended) A method according to claim 14 wherein said probe is having no more than five mismatches with a probe selected of a group composed of probes having a sequence TTATCCCCCTCTGATGGG (SEQ ID NO:5) or AGAGAAGCAAGCTTCTCGTCCG (SEQ ID NO:10) or GCCACTCCTCTTTCCGG (SEQ ID NO:7).
16. A method according to claim 11 wherein said character is clump-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysostaphin and/or Proteinase K.
17. (Amended) A method according to claim 16 further comprising hybridising said sample with at least one probe selected from a group consisting of probes capable of hybridising with nucleic acid found in *Staphylococcus aureus*, in *Staphylococcus haemolyticus*, in *Staphylococcus saprophyticus*.
18. A method according to claim 17 wherein said nucleic acid is ribosomal RNA.
19. (Twice Amended) A method according to claim 18 wherein said probe is having no more than five mismatches with a probe selected of a group consisting of probes having a sequence GCTAATGCAGCGCGGATCC (SEQ ID NO:8) or CCGAAGGGGAAGGCTCTA (SEQ ID NO:9) or AGAGAAGCAAGCTTCTCGTCCGTT (SEQ ID NO:10).

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20. (Twice Amended) A method according to claim 4 further comprising hybridising said sample with at least one positive control probe and/or with at least one negative control probe.

21. (Amended) A method according to claim 20 wherein said positive control probe comprising no more than five mismatches with a probe with the sequence GCTGCCTCCCGTAGGAGT (SEQ ID NO:11) and/or wherein said negative control probe comprises no more than five mismatches with a probe with the sequence ACTCCTACGGGAGGCAGC (SEQ ID NO:12).

22. (Twice Amended) A method according to claim 1 further comprising a one-step procedure of binding bacteria present in said sample to a microscopic slide and simultaneously fixing intracellular structures.

23. (Twice Amended) A method according to claim 1 wherein said probe is selected for its properties of reactivity with a selected one or more of bacterial genera and/or species including a consideration of the susceptibility to antibiotic treatment of said probe.

bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.

10. A method according to claim 9 wherein said character is of the rod type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme and/or Proteinase K.

(Amended) 11. A method according to claim 9 wherein said character is of the coccus type, further comprising determining a chain-like or clump-like character of said bacteria.

12. A method according to claim 11 wherein said character is chain-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.

(Amended) 13. A method according to claim 12 further comprising hybridising said sample with at least one probe selected from a group ^{consisting} of probes capable of hybridising with nucleic acid found in *Enterococcus faecalis*, in *Streptococcus pneumoniae*, in *Streptococcus mitis*, in *Streptococcus viridans*, in *Streptococcus sanguis*, in *Enterococcus faecium*.

14. A method according to claim 13 wherein said nucleic acid is ribosomal RNA.

(Amended) 15. A method according to claim 14 wherein said probe is having no more than five, [preferably no more than two] mismatches with a probe selected of a group composed of probes having a sequence ^(SEQ ID NO: 5) TTATCCCCCTCTGATGGG or ^(SEQ ID NO: 6) AGAGAAAGCAAGCTTCTCGTCCG or ^(SEQ ID NO: 7) GCCACTCCTTTTCCGG.

16. A method according to claim 11 wherein said character is clump-like, further comprising subjecting said sample to treatment with a lysis buffer comprising Lyostaphin and/or Proteinase K.

(Amended) 17. A method according to claim 16 further comprising hybridising said sample with at least one probe selected from a group ^{consisting} of probes capable of hybridising with nucleic acid found in *Staphylococcus aureus*, in *Staphylococcus haemolyticus*, in *Staphylococcus saprophyticus*.

18. A method according to claim 17 further comprising

Twice

(Amended)

19. A method according to claim 18 wherein said probe is having no more than five, [preferably no more than two]

20. A method according to [any of] claims 4 to 19 further comprising hybridising said sample with at least one positive control probe and/or with at least one negative control probe.

control probe. (Amended)
21. A method according to claim 20 wherein said positive control probe comprises no more than five mismatches with a probe with the sequence ^(SEQ ID NO: 11) GCTGCCCTCCCGTAGGAGT, and/or wherein said negative control probe comprises no more than five mismatches with a probe with the sequence ^(SEQ ID NO: 12) GCTGCCCTCCCGTAGGAGT.

ACTCCTACGGGAGGGAGC

22. A method according to [anyone of] claims [1 to 21] 1 further comprising a one-step procedure [to bind] bacteria present in said sample to a microscopic slide and

simultaneously fix^{ing} intracellular structures.

23. A method according to [anyone of] claims 1 to 22] 1 (DD) Amend A
wherein said probe is selected for its reactivity with (C)
one or [a group of] bacterial genera and/or species [having (C) C C C C
congruent] susceptibility to antibiotic treatment of said probe (C) C C

24. A probe detecting or identifying a bacterium in a sample, preferably a clinical sample, said probe designed to hybridise specifically with nucleic acid in bacteria with congruent susceptibility or resistance to antibiotics.

25. A probe according to claim 24 wherein said probe is (E)
having no more than five measurable

having no more than five, preferably no more than two mismatches with a probe selected of a group composed of

probes having a sequence GCCTGCCAGTTTCGAATGA of 5624D No. 1
(501PNV) 15

GTAGCCCTACTCGTAAGG or GAGCAAAGGTATTAACCTTACTCCC or
(SEQ ID No. 3)
(SEQ ID No. 4) (SEQ ID No. 5)

AGAGAAGCAAGCTCTCGTCCGA or (SEQ ID NO:7)
 (SEQ ID NO:8) CCCACTCCTCTTTTCCGGG or (SEQ ID NO:9)

ACAGAAGCAGCTCTCGTCGGT;

Cancelled
per [initials]

26. A diagnostic test kit comprising means for detecting or identifying a bacterium suspected of being present in a sample using a method according to anyone of claims 1 to 23 or using a probe according to claim 24 or 25.

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AMENDED SHEET